

A BIOSENSOR METHOD FOR DETECTION OF STAPHYLOCOCCAL ENTEROTOXIN A IN RAW WHOLE EGG*

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ABSTRACT

Staphylococcal enterotoxin A (SEA) is the most commonly recovered staphylococcal enterotoxin in food poisoning outbreaks. Our research objective was to develop a competitive immunoassay using a surface plasmon resonance (SPR) biosensor for the detection of SEA in raw eggs. Homogenized raw eggs were spiked with SEA and clarified by centrifugation at $14,989 \times g$. Anti-SEA was added to aliquots of the egg supernatants allowing SEA to bind with anti-SEA. The bound complex was separated from the free immunoglobulin G (IgG) by centrifugation. The supernatant was automatically sampled and injected over the SEA sensor surface of the SPR system. The IgG-bound response units were plotted against spiked SEA concentration. SEA was detected in whole egg at 1–40 ng/mL (ppb). The biosensor analysis including the sensor regeneration was 15 min per sample in an automated system. This biosensor assay can be utilized for SEA detection in liquid eggs.

INTRODUCTION

The food industry needs routine methods to detect trace levels of enterotoxins produced by *Staphylococcus aureus*. This organism produces enteric toxins that cause major foodborne gastroenteritis. Under temperature-abused conditions, the organism can grow in foods of animal origin. Heat processing

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and normal cooking temperatures can inactivate or kill the bacterial cells but the staphylococcal enterotoxins (SEs) are heat stable and are resistant to cooking and heating temperatures (Bergdoll 1979; Newsome 1988). Ten enterotoxins have been identified: A, B, C1, C2, C3, D, E (Bergdoll 1979), H (Su and Wong 1995), I, G (Munson *et al.* 1998) and J (Zhang *et al.* 1998). Staphylococcal enterotoxin A (SEA), B (SEB), C (SEC) and D (SED) are the most common in foods, and SEA is the most commonly recovered from food poisoning outbreaks.

The toxins have molecular weights ranging from 27,000 to 34,000. SEA has a molecular weight of 27,800 with an isoelectric point (pI) of 6.8 (Chu *et al.* 1966; Jay 2000). These toxins have relative thermostability of SEC > SEB > SEA (Tibana *et al.* 1987). The chemical and physical properties of these toxins were summarized by Jay (2000). The minimum level of enterotoxin to cause gastroenteritis in humans was approximately 1 ng/g or ng/mL of food (Noleto and Bergdoll 1982; Jay 2000), while Tatini *et al.* (1984) and Newsome (1988) reported <1 µg of toxin ingestion. Shantz *et al.* (1965) reported a toxic dose of 5 µg SEA and SEB when administered via intragastric administration. Methods with detection at or below 1 ng/g (1 ppb) are desired, and testing for both organism and toxin can assure the safety of processed foods.

The SEs were detected with immunochemical assays in the last 20 years. Wieneke (1991), Park *et al.* (1992, 1994), Bergdoll (1996) and Bennett (2005) described the principles and effectiveness of these methods. Su and Wong (1997) also reviewed the biological, immunological and polymerase chain reaction-based methods for the detection of SEs. There are very few reports on the detection of SEA in egg products. Yang *et al.* (2001) reported the use of a reverse passive latex agglutination (RPLA) method to detect SEA and SEB in scrambled and steamed eggs inoculated with *S. aureus*. Igarashi *et al.* (1985) also reported the detection of 4 ng of SEA in omelettes using the RPLA method.

Biosensor techniques offer a rapid, automated and multitoxin approach to detect these toxins in a food matrix. The principles and applications of the bioaffinity-based sensors, such as the surface plasmon resonance (SPR) biosensors, were described and reviewed by Malmquist and Karlsson (1997), Fivash *et al.* (1998), Nice and Catimel (1999) and Rich and Myska (2000). The Biacore (Piscataway, NJ) SPR biosensor allows direct real-time detection of the binding without chemically altering the structures of the ligands or analytes to generate signals. A capturing molecule is covalently immobilized to the sensor chip and the binding molecule is captured by the immobilized ligand in a continuous flow system. The mass of the captured molecule generates a change of the refractive index of the medium in the vicinity of the sensor. These changes are then detected by an optical system that measures the intensity and angle of the reflected light. These interactions are expressed in

arbitrary response units (RUs), which are monitored continuously and are plotted in real-time as RU versus time (in seconds).

The SPR biosensors have been utilized for the detection of SEA with sensitivities of 10–100 ng/mL (ppb). An IAsys SPR biosensor (Affinity Sensors, Paramus, NJ) detected SEA spiked in milk, hotdogs, mushrooms and potato salad at 10–100 ng/g samples (Rasooly and Rasooly 1999). The Biacore SPR biosensor was utilized for the detection of SEB in various food matrices. SEB was detected in spiked potted meat at 10–1000 ng/g and 1–1000 ng/mL in reconstituted dry milk with minimum detection of 10 ppb (Rasooly 2001). An improved detection of 2.5 ppb SEB spiked in ham tissues was reported by Medina (2003). Naimushin *et al.* (2002) reported the development of a miniature integrated two-channel SPR biosensor and detected 1 nM (28.4 µg/mL) SEB in seawater and 50 pM (1.42 µg/mL) in urine. Homola *et al.* (2002) developed a modulation-based SPR biosensor and showed detection of 5 ng/mL SEB. With a sandwich assay detection mode, the lowest detection limit was 0.5 ng/mL in buffer and milk samples. Strachan *et al.* (1997) reported an automated polymethacrylate particle-based immunosensor for the detection of SEB in cream at 5 ng/mL.

The objectives of the current study were to develop an SPR biosensor method for the detection of SEA in liquid egg at low nanogram levels, to optimize the detection of SEA in a competitive inhibition assay format, to optimize sample preparation of liquid egg for biosensor analysis of SEA and to improve the analytical throughput for routine analysis. Our long-term goal was to develop a multitoxin detection of SEs in foods.

MATERIALS AND METHODS

Equipment and Reagents

The Biacore upgraded with Biacore 1000 system software was equipped with BIAevaluation 2.1; the CM3 sensor chips, Surfactant P20, amine coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide (EDC), ethanolamine and Biacore sample tubes were from Biacore. SEA and the affinity-purified polyclonal sheep antibody against SEA were obtained from Toxin Technology (Sarasota, FL). The Tomy Refrigerated Micro Centrifuge MTX 150 was from Peninsula Laboratories (Belmont, CA) and the Multiblock heater was from Lab-Line Industries (Melrose Park, IL). *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (Hepes) (free acid), sodium azide, ethylenediaminetetraacetic acid (EDTA), sodium hydroxide and rabbit anti-SEA serum (Cat #S7656) were from Sigma Chemical Company (St. Louis, MO). ImmunoPure

Protein G Plus affinity gel and the immunoglobulin protein standard were from Pierce (Rockford, IL). Fresh shell eggs were purchased from local markets.

SPR Biacore Analysis

These studies were performed on an upgraded Biacore 1000 equipped with BIAlogue command software. The manufacturer's guidelines were followed for programming the methods preparation of the sensor surfaces, binding techniques and interpretation of the sensorgrams. The results from the real-time interactions of the ligand and the capturing molecule of the sensor were displayed in a sensorgram as optical response (RU) versus time (in seconds).

Affinity Purification of Anti-SEA Antibody

The reagents used were a 2-mL bed volume of ImmunoPure Protein G Plus affinity column; polyclonal anti-SEA serum; immunoglobulin G (IgG) binding buffer, Hepes-buffered saline (HBS) (pH 5.0), 10 mM Hepes (free acid), 150 mM NaCl, 3.4 mM EDTA (Na salt); elution buffer (1:1), 100 mM citrate (pH 3.0) and 100 mM HCl (pH 1.86); neutralization buffer, 100 mM Na₃PO₄ (pH 11.7); regeneration buffer which contained a 1:1 ratio of 100 mM citric acid (pH 3.0) and 100 mM HCl (pH 1.8); and Pierce immunoglobulin protein standard, 1.44 mg/mL. The calibration standard curve included 1.44, 0.72, 0.36, 0.18, 0.09 and 0 mg/mL in HBS.

The ImmunoPure Protein G Plus gel (2 mL) was packed in a 5-mL syringe barrel locked with a two-way stopcock attachment and plugged with a polystyrene frit with a medium porosity (70 μ m). Another frit was also inserted at the top of the gel. The column was preconditioned with five-column volumes of binding buffer. The antiserum (1 mL) was diluted with 3-mL binding buffer and quantitatively transferred to the preconditioned Protein G purification column. The serum was allowed to flow completely into the gel. The column was locked and the IgG was allowed to bind with Protein G for 30 min at room temperature (RT). The column was washed with 15 mL of the binding buffer, and the IgG was eluted with 10.5 mL of the elution buffer (citrate [pH 3.0] and 100 mM HCl, 1:1) into 4-mL polypropylene tubes. Typically, fraction 1 was a gel bed volume and eluted with 0.5 mL. The IgG fractions were eluted with 10 \times 1 mL eluting buffer, and each 1-mL fraction was neutralized with 0.75–1 mL 0.1 M Na₃PO₄ to a pH of around 6.5. The flow-through serum was again purified through the regenerated Protein G column to capture residual IgG.

For the determination of IgG purification efficiency, aliquots (20 μ L) from each fraction were transferred to microtiter wells and their protein contents were determined by the addition of 200 μ L Bio-Rad protein assay reagent

(Bio-Rad Laboratories, Hercules, CA). The positive protein fractions were screened as indicated by the formation of a blue color. The optical density was also measured in an enzyme-linked immunosorbent assay (ELISA) reader and quantified against the Pierce immunoglobulin protein standard. The fractions containing proteins were pooled into 15-mL graduated conical centrifuge tubes and the total volume was measured. The IgG protein content of the pooled fractions was measured by transferring $3 \times 20\text{-}\mu\text{L}$ aliquots to microtiter wells, and 200 μL of Bio-Rad protein reagent was added. After a 15-min incubation at RT, the optical density was measured at 595 nm in the Bio-Tek ELISA reader (Bio-Tek Instruments, Winooski, VT). The concentration of the IgG pooled fractions was calibrated against the Pierce immunoglobulin protein standard at 0, 0.9, 0.18, 0.36, 0.72 and 1.44 mg/mL.

Preparation of the SEA Sensor

The SEA was diluted with 10 mM sodium acetate buffer (pH 4.5) to a concentration of 0.5 mg/mL. A 200- μL aliquot of SEA was transferred to the Biacore sample tube and placed in the sample rack. The immobilization started with the conversion of the carboxymethyl groups on the dextran surface of the flow cells (FCs) on the sensor chips by activation with 10 μL of a mixture of equal volumes of NHS (115 mg/mL) and EDC (750 mg/mL). The carboxyl groups were converted to NHS esters with EDC-NHS, and these esters reacted spontaneously with the uncharged amino groups, which are favored by a pH below the pK_a or pI of the ligand. A 30- μL aliquot of the SEA preparation was automatically injected over the activated dextran. The remaining activated esters (not covalently bound with the IgG or toxin) on the dextran surface were inactivated (blocked) with 30- μL ethanolamine. The ligands and ethanolamine were injected in a flow rate of 3 $\mu\text{L}/\text{min}$. HBST (pH 6.8) containing 10 mM Hepes (free acid), 3.4 mM EDTA, 0.15 M sodium chloride and 0.005% (v/v) Biacore Surfactant P20 (Tween 20) were used as the running buffer.

Preparation of SEA Standards

SEA was diluted to 1 mg/mL with deionized water, aliquoted at 100 μL and stored at -60°C (stock A). A 100- μL volume of 1 mg/mL SEA was diluted with HBST buffer (pH 6.8) to 1 mL to a final concentration of 10 $\mu\text{g}/\text{mL}$ (stock B) and further aliquoted to 100 μL each. The working dilution of 100 ng/mL (ppb) SEA was prepared by diluting 100 μL of stock B with 9.9 mL HBS buffer. The SEA working standards (stock C) were serially diluted with HBS buffer (pH 6.8) from 100 to 1.56 ng/mL (ppb). Later experiments used standards by serially diluting 50 to 0.78, 25 to 0.39 and 20 to 0.31 ng/mL.

Characterization of the SEA Sensor

The binding characteristics of the SEA sensors were evaluated with Protein G-purified anti-SEA utilizing HBST buffer (pH 6.8) as running buffer. This pH allowed the protonation of SEA, which had a reported pI of pH 7.3 (Anon 2003). Anti-SEA (200 μ L) with concentrations of 0, 0.1, 0.25 and 0.5 mg/mL was transferred to the Biacore sample tubes. The analysis consisted of an injection of 15 μ L of the antibody mixture in a flow rate of 3 μ L/min. The bound complex was desorbed from the SEA sensor with 5 μ L of 100 mM HCl in a flow rate of 5 μ L/min. The RU responses of the anti-SEA were determined from the capture of the 200 μ L of 0.1, 0.25 and 0.5 mg/mL anti-SEA in HBST buffer. The antibody concentration with >1000 RU response was utilized for the analysis of spiked egg samples.

SEA Analysis in Raw Whole Egg

SEA was analyzed in spiked egg supernatant. A shell egg was broken and the egg was manually mixed with a spatula. Ten-gram aliquots were centrifuged at 13,500 rpm ($14,898 \times g$) and the supernatant was decanted into another tube. Aliquots of 180 μ L were transferred to Biacore tubes and spiked with 20 μ L of 0, 10, 25, 50, 100, 200 and 400 ppb SEA resulting in a final concentration of 0, 1, 2.5, 5, 10, 20 and 40 ppb SEA. The samples were mixed with a vortex mixer and incubated overnight to equilibrate. Anti-SEA (10 μ L of 1 mg/mL) was added to the egg samples and allowed to incubate for 1.5 h at RT.

SEA was analyzed in spiked whole egg. A shell egg was broken and placed in a 100-mL beaker and sonicated for 5 min with 50% pulse (power #4). Aliquots (1 g) of homogenized egg were weighed into v-bottom polypropylene tubes (12×72 mm). The egg samples were spiked with 100 μ L of 0, 10, 50, 100, 200 and 400 ppb SEA resulting in 0, 1, 5, 10, 20 and 40 ppb final concentration. The samples were equilibrated for 1 h at 4C and centrifuged at 13,500 rpm ($14,989 \times g$) for 10 min. The supernatants were decanted and 200- μ L aliquots were transferred to the Biacore sample tubes. Ten or twenty microliters of anti-SEA (1 mg/mL) was added to each sample tube except for the HBST blank and egg blank samples. The samples were incubated for another 2 h at RT and centrifuged at 6000 rpm ($2961 \times g$) for 10 min to separate the SEA and anti-SEA IgG complex. The samples were loaded onto the Biacore system and analyzed without separating the supernatant. Injection of 15- μ L samples (3 μ L/min flow rate) over the toxin sensor surface allowed the SEA sensor to capture the excess anti-SEA in the samples. The bound anti-SEA was measured 60 s after injection. The sensor surface was regenerated by desorbing with two pulses of 5 μ L of 100 mM HCl at 5 μ L/min flow rate.

RESULTS AND DISCUSSION

Affinity Purification of Anti-SEA Immunoglobulin

One milliliter of rabbit anti-SEA serum purified in an ImmunoPure Protein G Plus column yielded 3.73 mg/mL IgG in a total pooled fractions of 9.5 mL. The total IgG yield was 35.43 mg/mL serum when calibrated against the Pierce immunoglobulin protein standard. This affinity purification procedure yielded 99% IgG in a single pass. Its binding to SEA sensor is described in the next paragraph. The use of this affinity-purified anti-SEA in this study was necessary because the commercially available affinity-purified anti-SEA had low binding to the SEA sensor yielding low signals and its use in high concentrations was costly. Our affinity-purified Sigma anti-SEA can be used for 950 sample analyses.

SEA Sensor Surface

A typical sensorgram of the immobilized SEA (0.5 mg/mL) is shown in Fig. 1. The carboxyl groups on the dextran surface of the Biacore CM3 sensor chip were activated with NHS and carbodiimide (EDC) for the covalent linkage of the SEA. The immobilization contact time was 10 min (injecting 30 μ L at 3 μ L/min). The excess NHS-ester active sites were blocked with ethanolamine. The resulting resonance signals of the immobilized SEA ligands were 2224.1 and 2438.6 RUs for SEA sensors I and II, respectively, and equivalent to 12.01 and 13.17 ng SEA/mm² surface. The SEA surface (in nanograms per millimeter squared) was determined as: RU SEA/1000 \times 5.40 factor. This factor was a ratio of the molecular weight of IgG to SEA (150,000/27,800 = 5.40). Karlsson *et al.* (1991) and Fagerstram and O'Shannessy (1993) reported that the sensor surface protein load of 1 ng IgG/mm² generated 1000 RU of SPR signal. The CM3 chip (formerly Pioneer Chip F1) has shorter dextran surface molecules compared to the CM5 standard chip. These sensor chips were evaluated for their binding to anti-SEA at concentrations of 0.1, 0.25 and 0.5 mg/mL IgG, and the results are shown in Fig. 2. SEA sensor chip I generated higher RU than SEA sensor chip II. The antibody concentration with >1000 RU binding to the sensor was utilized for the analysis of SEA in the egg samples.

SEA Analysis in Spiked Whole Egg

The supernatant of the egg samples centrifuged at $14,898 \times g$ was spiked with 0, 1–40 ppb SEA. The spiked samples were equilibrated for 1 h or overnight at 4C and centrifuged at $2961 \times g$ to separate the bound complex (SEA/anti-SEA IgG). The centrifuged samples were analyzed with the Biacore

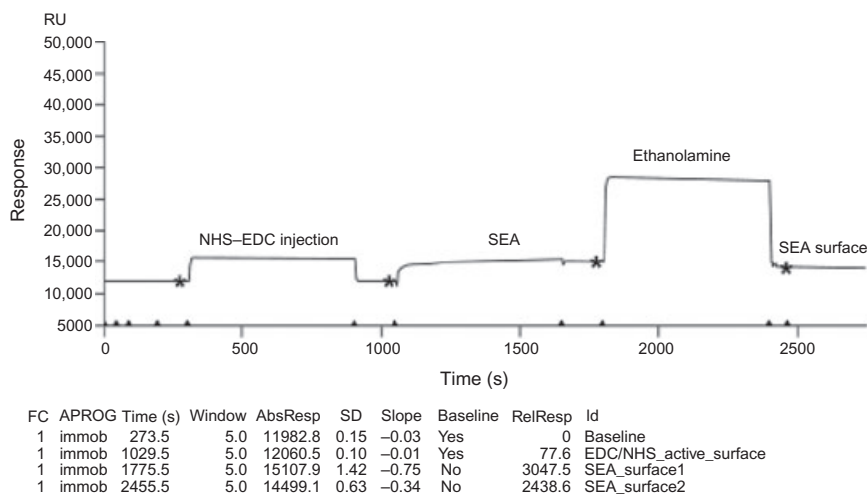


FIG. 1. PREPARATION OF STAPHYLOCOCCAL ENTEROTOXIN A (SEA) SENSOR

A real-time sensorgram of SEA (30 μ L of 0.5 mg/mL) immobilization on flow cell (FC) 1 of the CM3 sensor chip at 3 μ L/min flow rate. The carboxyl groups of the dextran surface were converted to *N*-hydroxysuccinimide (NHS) esters (273.5–1029.5 s) and covalently bound to the free amino groups of the SEA (1029.5–1775.5 s). The residual active esters were blocked with ethanolamine (1775.5–2455.5 s). The resulting SEA surface net response units (RUs) was 2438.6 and was equivalent to 13.17 ng SEA/mm². The surface plasmon resonance biosensor detected the “real-time” change (in seconds) of the refractive index (indicated by RU) resulting from the binding of SEA molecules to the dextran surface. EDC, *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide.

system without decanting the supernatant from the pellet. We have reported that in the analysis of SEB in ham extracts (Medina 2003) and in milk samples (Medina 2005), SEB and anti-SEB IgG remained in the supernatant after centrifugation at 2961 \times g. Figure 3 shows the dose response curves in five trials when 0, 1–40 ppb SEB spiked in whole egg supernatant and in homogenized egg was plotted against RU of excess and unbound anti-SEA. In trials I–III, 10 μ g anti-SEA was added to a 1-g sample. Because of lower anti-SEA binding signals in trial III, the anti-SEA concentration was increased to 20 μ g/g egg sample (20 μ L of 1 mg/mL). In trial III, 673 RUs were generated compared to a mean of 874 in the other trials. Table 1 summarizes the percentage binding or competition of the SEA with the antibody binding sites where the excess antibody sites bound to the SEA sensor. Using the RU of the “0” control (egg sample plus anti-SEA) as 100% binding, the RU responses of the spiked samples were compared. The RU responses of the “0” control in trials I, II, IV and V were 939, 843, 887 and 826, respectively. The samples containing 1 ppb had a displacement of 5% (SD was 3.1), while those spiked with 40 ppb SEA had a mean bound of 86.9% with displacement of 13.1%.

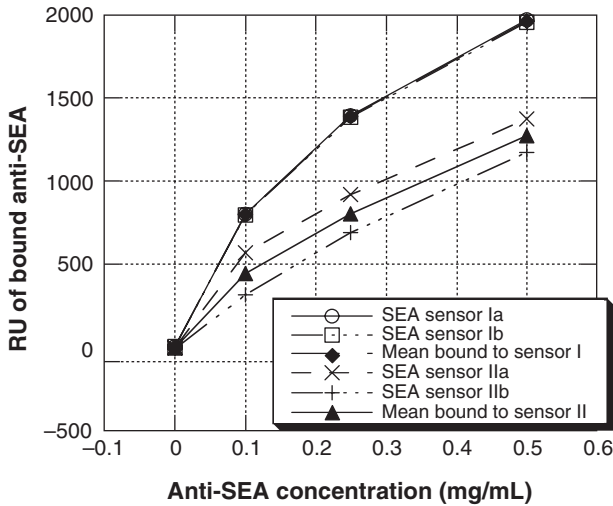


FIG. 2. EVALUATION OF THE STAPHYLOCOCCAL ENTEROTOXIN A (SEA) SENSORS WITH 0, 0.1, 0.25 AND 0.5 mg/mL ANTI-SEA AT 15 MIN OF CONTINUOUS FLOW BINDING. Sensor II generated lower antibody binding than with sensor I, but the SEA surfaces were 13.17 and 12.01 ng SEA/mm², respectively. RU, response unit.

The nonspecific binding (NSB) interferences from the egg samples were 63–70% except for the NSB in trial III. The NSB RUs of the egg samples in trials I–V were 655, 600, 560, 556 and 532, respectively. The binding responses of the “blank” egg samples were approximately 200 RU below the lowest RU responses of samples spiked with 40 ppb SEA. In future assays, the selection of antibody concentration must generate at least a 200 RU difference with the egg blank control and the highest concentration of the spiked SEA.

The displacements shown in Table 1 are lower than those reported for the analysis of SEB in milk (Medina 2005), where the spiked SEB was detected from 0.312–25 ppb. Presumably, there are two reasons for these differences. First, the egg sample preparation was minimal using only centrifugation compared to subjecting the milk samples to heating at 95°C and centrifugation to remove potential interfering substances. Heating or acidification of the egg samples precipitated the eggs and entrapped the liquid components, thus, recovering SEA was not easily feasible. Second, the anti-SEB had higher avidity and affinity. SEB is a superantigen compared to SEA, thus SEB is produced with higher avidity and affinity. Anti-SEB was utilized in these assays in much lower concentration than the anti-SEA.

In this study, centrifugation of the homogenized egg at $14,898 \times g$ was sufficient to clarify the egg samples reducing sample interferences and allowed SEA to remain in the supernatant. Bennett and McClure (1980) reported in a

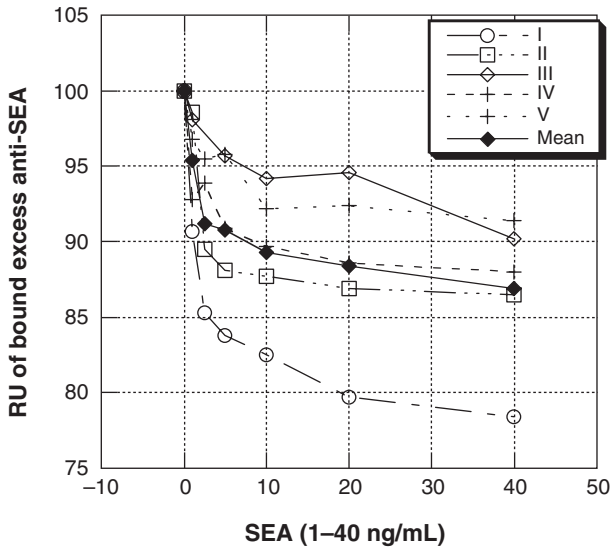


FIG. 3. DOSE RESPONSE CURVES OF STAPHYLOCOCCAL ENTEROTOXIN A (SEA) SPIKED IN LIQUID WHOLE EGGS

Response unit (RU) responses from the capture of anti-SEA spiked in whole liquid egg in five trials. Sensor I was utilized for trials I and II, while sensor II was utilized for trials III–V. Egg samples were spiked with 0, 1, 2.5, 5, 10, 20 and 40 ppb SEA, centrifuged at $14,989 \times g$. The supernatants were incubated with anti-staphylococcal enterotoxin A, centrifuged ($2961 \times g$) to separate the bound SEA–immunoglobulin G complex and analyzed with the Biacore (Piscataway, NJ) system with a 5-min contact time at $3 \mu\text{L}/\text{min}$. In trials I–III, $10 \mu\text{g}$ anti-SEA/g sample was used but because of lower signals in trial III, the anti-SEA concentration was increased to $20 \mu\text{g}/1 \text{ g}$ egg sample. The SEA sensor was regenerated with 100 mM HCl.

collaborative study on the extraction and separation of SEs that centrifugation at $32,800 \times g$ allowed the SE to remain in the supernatant. Park *et al.* (1994) also separated SEB from extracts by centrifugation at $16,300 \times g$. Rasooly and Rasooly (1999) and Rasooly (2001) centrifuged the samples at 1000 and $14,000 \times g$, respectively, and SEB remained in the supernatant.

CONCLUSION

A rapid and sensitive method for the detection of SEA was developed using a competitive immunoassay and analyzing with a Biacore SPR biosensor. We have optimized the sample preparation for the biosensor analysis of whole egg samples. The whole shell egg was homogenized with a sonicator and centrifuged at $14,898 \times g$ for clarification. The competitive immu-

TABLE 1.
ANALYSIS OF STAPHYLOCOCCAL ENTEROTOXIN A (SEA)
SPIKED IN LIQUID WHOLE EGG

SEA in sample	I	II	III	IV	V	Mean	SD
HBS	1.5	2.4	0	0.07	0		
0	100	100	100	100	100	100	
1	90.7	98.6	98.1	92.8	96.8	95.4	3.1
2.5	85.3	89.5	NA	93.9	95.5	91.2	4.0
5	83.8	88.1	95.7	90.9	95.8	90.8	4.6
10	82.5	87.7	94.2	89.7	92.2	89.3	4.0
20	79.7	86.9	94.6	88.6	92.4	88.4	5.1
40	78.4	86.5	90.2	88.0	91.4	86.9	4.6
Egg blank*	69.7	70.2	83.2	62.7	64.4	70.0	7.2
$\Delta 40$ ppb blank	8.7	16.3	7	26	27	16.9	

*Egg sample without SEA or anti-SEA.

Results are shown as percentage displacement of excess immunoglobulin G binding with the SEA sensor by the SEA spiked in samples where % displacement = response units (RUs) binding of sample/RU binding "0" sample (with anti-SEA but no toxin) \times 100. HBS, *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid-buffered saline; NA, not analyzed.

noassay format consisted of allowing the SEA in the samples to bind with anti-SEA IgG and the excess IgG was captured by the SEA sensor. The bound complex was separated from the free IgG by centrifugation at $2961 \times g$ prior to the Biacore analysis. This assay format resulted in the detection of SEA from 1–40 ng/mL in spiked liquid egg. The sample preparation was <1 h for 10 samples plus a 2-h incubation of sample SEA and anti-SEA. The Biacore analysis was completed in 15 min per sample and each SEA sensor (one FC) was utilized in 96–122 analyses. The cost of analysis (sensor chip, SEA, anti-SEA) was <\$3.00 per sample. This assay can be used to validate results by other commercial screening assays for SEA detection in eggs. The biosensor analysis is fully automated and it is anticipated that this method will be utilized for multitoxin detection in various food matrices.

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REFERENCES

- ANONYMOUS. 2003. *Product Literature, Toxin Technology*. Toxin Technology, Sarasota, FL.
- BENNETT, R.W. 2005. Staphylococcal enterotoxin and its rapid identification in foods by enzyme linked immunosorbent assay-based methodology. *J. Food Prot.* 68, 1264–1270.
- BENNETT, R.W. and MCCLURE, F. 1980. Extraction and separation of staphylococcal enterotoxin in foods: Collaborative study. *J. AOAC Int.* 63, 1205–1210.
- BERGDOLL, M.S. 1979. Staphylococcal intoxications. In *Food-borne Infections and Intoxications*, 2nd Ed. (H. Reimann and F.L. Bryan, eds.) pp. 443–490, Academic Press, New York, NY.
- BERGDOLL, M.S. 1996. Detection of the staphylococcal toxins. *Adv. Exp. Med. Biol.* 391, 465–479.
- CHU, F.S., THADHANI, K., SCHANTZ, E.J. and BERGDOLL, M.S. 1966. Purification and characterization of staphylococcal enterotoxin A. *Biochemistry* 5, 3281–3289.
- FAGERSTRAM, L.G. and O'SHANNESY, D.J. 1993. Surface plasmon resonance detection in affinity technologies In *Handbook of Affinity Chromatography*, Chromatographic Series (T. Kline, ed.) pp. 229–252, Marcel Dekker, New York, NY.
- FIVASH, M., TOWLER, E.M. and FISHER, R.J. 1998. BIAcore for macromolecular interaction. *Curr. Opin. Biotech.* 9, 97–101.
- HOMOLA, J., DOSTALEK, J., CHEN, S., RASOOLY, A., JIANG, S. and YEE, S.S. 2002. Spectral surface plasmon resonance biosensor for detection of staphylococcal enterotoxin B in milk. *Int. J. Food Microbiol.* 75, 61–69.
- IGARASHI, H., FUJIKAWA, H., SHINGAKI, M. and BERGDOLL, M.S. 1985. Detection of staphylococcal enterotoxins in food poisoning outbreaks by reversed passive latex agglutination. In *The Staphylococci*, Zbl. Bakt. Suppl 14. (J. Jcljaszwicz, ed.) pp. 255–257, Gustav Fisher Verlag, Stuttgart, New York.
- JAY, J.M. 2000. *Modern Food Microbiology*, 6th Ed., pp. 441–459, Aspen Publishers, Gaithersburg, MD.
- KARLSSON, R.M., MICHAELSON, A. and MATTSON, L.J. 1991. Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *Immunol. Methods* 145, 229–240.
- MALMQUIST, M. and KARLSSON, R. 1997. Biomolecular interaction analysis: Affinity biosensor technologies for functional analysis of proteins. *Curr. Opin. Chem. Biol.* 1, 378–383.

- MEDINA, M.B. 2003. Detection of staphylococcal enterotoxin B (SEB) with surface plasmon resonance biosensor. *J. Rapid Methods Autom. Microbiol.* 11, 225–243.
- MEDINA, M.B. 2005. A biosensor method for a competitive immunoassay detection of staphylococcal enterotoxin B (SEB) in milk. *J. Rapid Methods Autom. Microbiol.* 13, 37–55.
- MUNSON, S.H., TREMAINE, M.T. and BETLEY, M.J. 1998. Identification and characterization of staphylococcal enterotoxin types G and I from *Staphylococcus aureus*. *Infect. Immun.* 66, 3337–3348.
- NAIMUSHIN, A.N., SOELBERG, S.D., NGUYEN, D.K., DUNLAP, L., BARTHOLOMEW, D., ELKIND, J., MELENDEZ, J. and FURLONG, C.E. 2002. Detection of *Staphylococcus aureus* enterotoxin B at femtomolar levels with a miniature integrated two-channel surface plasmon resonance (SPR) sensor. *Biosens. Bioelectron.* 17, 573–584.
- NEWSOME, R.L. 1988. *Staphylococcus aureus*. *Food Technol.* 42(4), 192–195.
- NICE, E.C. and CATIMEL, B. 1999. Instrumental biosensors: New perspectives for the analysis of biomolecular interactions. *BioEssays* 21, 339–352.
- NOLETO, A.L. and BERGDOLL, M.S. 1982. Production of enterotoxin by *Staphylococcus aureus* strain that produces identifiable enterotoxins. *J. Food Prot.* 45, 1096–1097.
- PARK, C.E., AKHTAR, M. and RAYMAN, M.K. 1992. Nonspecific reactions of a commercial enzyme-linked immunosorbent assay kit (TECRA) for detection of staphylococcal enterotoxins in foods. *Appl. Environ. Microbiol.* 58, 2509–2512.
- PARK, C.E., AKHTAR, M. and RAYMAN, M.K. 1994. Evaluation of a commercial enzyme immunoassay kit (RIDASCREEN) for detection of staphylococcal enterotoxins A, B, C, D and E in foods. *Appl. Environ. Microbiol.* 60, 677–681.
- RASOOLY, A. 2001. Surface plasmon resonance analysis of staphylococcal enterotoxin B in food. *J. Food Prot.* 64, 37–43.
- RASOOLY, L. and RASOOLY, A. 1999. Real time biosensor analysis of staphylococcal enterotoxin A in food. *Int. J. Food Microbiol.* 49, 119–127.
- RICH, R.L. and MYSKA, D.G. 2000. Advances in surface plasmon resonance biosensor analysis. *Curr. Opin. Biotech.* 11, 54–61.
- SHANTZ, E.J., ROESSLER, W.G., WAGMAN, J., SPERO, L., DUNNERY, D.A. and BERGDOLL, M.S. 1965. Purification of staphylococcal enterotoxin B. *Biochemistry* 4, 1011.
- STRACHAN, N.J.C., JOHN, P.G. and MILLAR, I.G. 1997. Application of a rapid automated immunosensor for the detection of *Staphylococcus aureus* enterotoxin B in cream. *Int. J. Food Microbiol.* 35, 293–297.

- SU, Y-C. and WONG, A.C.L. 1995. Identification and purification of a new enterotoxin H. *Appl. Environ. Microbiol.* *61*, 1438–1443.
- SU, Y-C. and WONG, A.C.L. 1997. Current perspectives on detection of staphylococcal enterotoxins. *J. Food Prot.* *60*, 195–202.
- TATINI, S.R., HOOVER, D.G. and LACHICA, R.V.F. 1984. Methods for the isolation and enumeration of *S. aureus*. In *Compendium of Methods for the Microbiological Examination of Foods*, 2nd Ed. (M.L. Speck, ed.) p. 411, American Society for Microbiology, Washington, DC.
- TIBANA, A., RAYMANM, K. and AKHTAR, M. 1987. Thermal stability of staphylococcal enterotoxins A, B and C in a buffered system. *J. Food Prot.* *50*, 239–242.
- WIENEKE, A.A. 1991. Comparison of four kits for the detection of staphylococcal enterotoxin in foods from outbreaks of food poisoning. *Int. J. Food Microbiol.* *14*, 305–312.
- YANG, S.E., YU, R.C. and CHOU, C.C. 2001. Influence of holding temperature on the growth and survival of *Salmonella* spp. and *Staphylococcus aureus* and the production of staphylococcal enterotoxin in egg products. *Int. J. Food Microbiol.* *63*, 99–107.
- ZHANG, S., IANDOLO, J. and STEWART, G.C. 1998. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (*SEJ*). *FEMS Microbiol. Lett.* *168*, 227–233.